# EXHIBIT B
# CLEAN VERSION OF THE SUBSTITUTE SPECIFICATION

# TITLE OF THE INVENTION

PROTEINS HAVING EFFECTS OF CONTROLLING CELL MIGRATION AND CELL DEATH

## CROSS REFERENCE TO RELATED APPLICATIONS

This application is a Continuation-in-part of International Patent Application No. PCT/JP02/07676 filed July 29, 2002 and published on March 6, 2003 as WO 03/018804, claiming priority to Japanese application 2001-256910 filed August 27, 2001. Each of the above applications, and each document cited in this text and in each of the above applications ("application cited documents") and each document cited or referenced in each of the application cited documents, and any manufacturer's specifications or instructions for any products mentioned in this text and in any document incorporated into this text, are hereby incorporated herein by reference; and, technology in each of the documents incorporated herein by reference can be used in the practice of this invention.

It is noted that in this disclosure, terms such as "comprises", "comprised", "comprising", "contains", "containing" and the like can have the meaning attributed to them in U.S. Patent law; e.g., they can mean "includes", "included", "including" and the like. Terms such as "consisting essentially of" and "consists essentially of " have the meaning attributed to them in U.S. Patent law, e.g., they allow for the inclusion of additional ingredients or steps that do not detract from the novel or basic characteristics of the invention, i.e., they exclude additional unrecited ingredients or steps that detract from novel or basic characteristics of the invention, and they exclude ingredients or steps of the prior art, such as documents in the art that are cited herein or are incorporated by reference herein, especially as it is a goal of this document to define embodiments that are patentable, e.g., novel, nonobvious, inventive, over the prior art, e.g., over documents cited herein or incorporated by reference herein. And, the terms "consists of" and "consisting of" have the meaning

ascribed to them in U.S. Patent law; namely, that these terms are closed ended.

## FIELD OF THE INVENTION

The present invention relates to a protein that has effects of controlling cell migration and cell death of neurons and the like, a DNA that encodes the protein, control of cell migration and/or cell death, and a method for screening a promoter or an inhibitor of the effects of controlling cell migration and/or cell death, by using the protein and the like.

## BACKGROUND OF THE INVENTION

More than one hundred billion neurons exist in human brain to form complex neural circuits. Only prescribed numbers of them are formed in the adequate positions as development progresses. These neurons have very complicated shapes which never be seen in other somatic cells and extend two kinds of processes dendrite and axon from a cell body which is protoplasm including a nucleus. A dendrite comprises numerous thorn structures called spine and forms postsynaptic region that has a function for receiving information from other cells. It is known that this neuron specific shape is determined by a neuron specific actin-binding protein.

On the other hand, brain is an important organ that controls not only the action at unconsciousness level but also what is called higher-order function such as emotion, memory, learning, and creation. However, it has not revealed yet how the regions in brain are determined and how the differentiation of brains that is specific in each region are occurred. Neuronal migration is essential for construction of brain tissue, for example in cerebral cortex, a layer structure is formed by division of neural stem cells (radial glial cell) at ventricular zone and radial migration thereof with the help of the radial processes inherited in division. Although it has been indicated that molecules such as PS-NCAM or Slit are involved in these migrations of neurons, the relation has been hardly revealed yet.

As aforementioned, radial migration of postmitotic neurons is essential for neocortical development (J. Comp. Neurol. 145, 61-83, 1972, Nat. Neurosci. 4, 143-150, 2001, Nature 409, 714-720, 2001). Neurons generated in the ventricular zone have to make at least two important decisions in order to reach their destination correctly: when to start and where to stop migration. The stop of migration is thought to be regulated by Reelin (Nature 374, 719-723, 1995, Nature 389, 730-733, 1997, Nature 389, 733-737, 1997, Neuron 24, 471-479, 1999, Neuron 24, 481-489, 1999, Cell 99, 635-647, 1999, Cell 97, 689-701, 1999, Neuron 27, 33-44, 2000), however, the molecule relating to the start of migration has been poorly understood. An exception has been reported that disruption of an actin-binding protein Filamin 1 results in a human neuronal migration disorder, periventricular nodular heterotopia, in which many neurons remain lining the ventricular surface (Neuron 16, 77-87, 1996, Neuron 21, 1315-1325, 1998).

The present invention relates to a protein of the effects of controlling cell migration and cell death of such as neurons and a DNA encoding the protein, particularly, an object of the present invention is to provide a method of controlling cell migration and/or cell death and a method of screening a promoter or an inhibitor of the effects of controlling cell migration and/or cell death with the use of proteins controlling the cell motility and cell death of neurons and the DNA encoding the proteins by interacting an actin-binding protein and promoting the degradation of the actin-binding protein.

Analysis of the cerebral cortex having disorder in layer structure is thought to provide an important clue for clarification of molecular mechanism relating to neuronal migration during the development of cerebral cortex, for instance the clarification of molecular mechanism which arrests cell migration is progressing rapidly by the study of reeler mouse. Likewise, periventricular nodular heterotopia, in which immovable neurons remain at neuroepithelial layer is thought to be another clue for solving the mechanism for starting/maintaining the migration of neurons, and abnormality of an actin-

binding protein Filamin 1 has been revealed to be a cause. (Though "Filamin 1" is sometimes called "Filamin A", it is indicated "Filamin 1" in the present invention.)

Meanwhile, the inventors reported about a rat nascent stage cerebral cortex-derived cytoskelton-associated novel protein FILIP (Filamin-interacting protein), it was predicted that the FILIP (S-FILIP) molecule comprised 965 amino acid residues in total, and revealed that it comprised coiled-coil structure including leucine zipper motifs at N-terminal-half of the molecule. Moreover, yeast two-hybrid screening or immunoprecipitaion analyses revealed that the C-terminal-half of FILIP molecule is combined with an actin-binding protein, Filamin 1. Filamin 1 is an essential molecule for cell migration during cerebral cortex formation period, and it is known that mutation of Filamin 1 gene causes periventricular nodular heterotopia characterized in migration disorder of cerebral cortical neuron. This led to the possibility that FILIP (S-FILIP) controls cell migration by associating with Filamin 1 to control those function at developing cerebral cortex. To verify this hypothesis, FILIP was expressed in a cultured cell and the aspect of cell migration was observed with time following. In consequence, migration of FILIP-expressing cell was controlled compared to the control, FILIP (S-FILIP) was indicated as a negative control factor of cell migration.

Subsequently, as the result of a keen study by the present inventors, FILIPs (L-FILIP and S-FILIP) were identified, FILIPs were found that they had functions for controlling cell motility and cell death, and the present invention was completed. That is, FILIP molecule (965 amino acid residues;S-FILIP;GenBank accession number D87257)( SEQ ID NOS: 3 and 4 in sequence listing) and L-FILIP which comprises 1212 residues, being constructed by adding 247 residues to molecule on the N terminal side (GenBank accession number AB055759)( SEQ ID NOS: 1 and 2 in the sequence listing).

Moreover, the result of a further study by the present inventor, human FILIP molecule (1213 amino acid residues; h-FILIP; -GenBank accession

number AB086011) (SEQ ID NOS: 5 and 6 in the sequence listing), which is a human orthologue of mouse L-FILIP, was identified from human DNA library.

The present inventors found that when the novel protein L-FILIP or S-FILIP was introduced into cells, these molecules partially coexisted with filamentous-actin within the cells, and in the same cell, the degradation of filamentous-actin was yielded, it became smaller and shorter, the lamellipodia formation ratio from cell membrane was decreased, and the cell migration ratio was significantly decreased. They also found that L-FILIP which is a novel molecule had more significant Filamin 1 degradation promoting effect as well as it expressed more protein at cerebral cortex neuroepithelium than S-FILIP, from the result of investigation using cultured cells. These facts revealed that S-FILIP but L-FILIP mainly plays the role of controlling cell migration negatively by promoting degradation of Filamin 1 at cerebral cortex neuroepithelium.

When S-FILIP or L-FILIP and Filamin 1 were expressed in the same cell, the change in Filamin 1 was observed, and the degradation of Filamin 1 progressed by expression of FILIP was observed similarly as aforementioned. These changes were also significant at L-FILIP. When the expression of Filamin 1 at the brain of normal rats during their fatal stage was examined, expression of Filamin 1 gene was observed, while a number of cells were observed of which expression amount of Filamin 1 protein had largely decreased in cells localized in ventricular zone, where expression of FILIP gene being observed, and cell migration toward cortical plate having not yet occurred. On the other hand, reduction of the cell number was identified in the cultured cell to which novel molecule L-FILIP was introduced, and it was revealed that FILIPs were also related to the control of cell death. The present invention was completed based upon the knowledge mentioned above.

## DESCRIPTION OF THE INVENTION

For the purposes of the present application, the term "DNA" is intended

to include an isolated DNA molecule.

The present invention relates to: an isolated DNA that encodes a protein described in the following (a) or (b): (a) a protein that comprises an amino-acid sequence shown in SEQ ID NO: 2 in the sequence listing, and (b) a protein which comprises an amino-acid sequence wherein 1 or several amino acids are deleted, substituted or added in an amino-acid sequence shown in SEQ ID NO: 2 in the sequence listing, and has effects of controlling cell migration and cell death (paragraph 1); a DNA that comprises a base sequence shown in SEQ ID NO: 1 in the sequence listing, complementary sequence thereof, or a sequence comprising part or whole of these sequences (paragraph 2); a DNA that hybridizes with the DNA consisting of the gene according to paragraph 2 in stringent condition and encodes the proteins having the effects of controlling cell migration and cell death (paragraph 3); a DNA that encodes the protein described in the following (a) or (b):(a) a protein that comprises an amino-acid sequence shown in SEQ ID NO: 4 in sequence listing, and (b) a protein that comprises an amino-acid sequence wherein 1 or several amino acids are deleted, substituted, or added in an amino-acid sequence shown in SEQ ID NO: 4 in the sequence listing, and has effects of controlling cell migration and cell death (paragraph 4); a DNA that comprises the base sequence shown in SEQ ID NO: 3 in sequence listing, complementary sequence thereof, or a sequence comprising part or whole of these sequences (paragraph 5); and a DNA that hybridizes with the DNA consisting of the gene according to paragraph 5 in stringent condition and encodes the protein having the effects of controlling cell migration and cell death (paragraph 6).

The present invention also relates to: a DNA that encodes the protein described in the following (a) or (b); (a) a protein that comprises an amino-acid sequence shown in SEQ ID NO: 6 in the sequence listing, and (b) a protein that comprises an amino-acid sequence wherein 1 or several amino acids are deleted, substituted, or added in SEQ ID NO: 6 in the sequence listing, and has effects of controlling cell migration and cell death (paragraph 7); a DNA that

comprises the base sequence shown in SEQ ID NO: 5 in the sequence listing, complementary sequence thereof, or a sequence comprising part or whole of these sequences (paragraph 8); a DNA that hybridizes with the DNA consisting the gene according to paragraph 8 in stringent condition and encodes the protein having the effects of controlling cell migration and cell death (paragraph 9); a protein that comprises the amino-acid sequence shown in SEQ ID NO: 2 in the sequence listing (paragraph 10); a protein that comprises an amino-acid sequence wherein 1 or several amino acids are deleted, substituted, or added in the amino-acid sequence shown in SEQ ID NO: 2 in the sequence listing, and has effects of controlling cell migration and cell death (paragraph 11); a protein that comprises the amino-acid sequence shown in SEQ ID NO: 4 in the sequence listing(paragraph 12); a protein that comprises an amino-acid sequence wherein 1 or several amino acids are deleted, substituted, or added in the amino-acid sequence shown in SEQ ID NO: 4 in the sequence listing, and has effects of controlling cell migration and cell death (paragraph 13); a protein that comprises amino-acid sequence shown in SEQ ID NO: 6 in the sequence listing (paragraph 14); a protein that comprises an amino-acid sequence wherein 1 or several amino acids are deleted, substituted, or added in the amino-acid sequence shown in SEQ ID NO: 6 in the sequence listing, and has effects of controlling cell migration and cell death (paragraph 15); the protein according to paragraph 11, 13,or 15, wherein control of cell migration and cell death is caused by the degradation of Filamin 1 (paragraph 16).

The present invention further relates to: a peptide that comprises a part of the protein according to any one of paragraphs 10 to 16, and has effects of controlling cell migration and cell death (paragraph 17); the peptide according to paragraph 17, wherein control of cell migration and cell death is caused by the degradation of Filamin 1 (paragraph 18); a fusion protein or a fusion peptide wherein the protein according to any one of paragraphs 10 to 16, or the peptide according to paragraph 17 or 18 is bound to a marker protein and/or a peptide tag (paragraph 19); an antibody that specifically binds to the protein

according to any one of paragraphs 10 to 16 or the peptide according to paragraph 17 or 18 (paragraph 20); the antibody according to paragraph 20, wherein the antibody is a monoclonal or a polyclonal antibody (paragraph 21); a recombinant protein or a recombinant peptide to which the antibody according to paragraph 20 or 21 specifically binds (paragraph 22); a host cell that comprises expression system which capable of expressing the protein according to any one of paragraphs 10 to 16 or the peptide according to paragraph 17 or 18 (paragraph 23); a non-human animal whose a gene function encoding the protein according to any one of paragraphs 10 to 16 or the peptide according to paragraph 17 or 18 is deficient on its chromosome (paragraph 24); a non-human animal that over-expresses the protein according to any one of paragraphs 10 to 16 or the peptide according to paragraph 17 or 18 (paragraph 25); and the non-human animal according to paragraph 24 or 25 which is a mouse or a rat (paragraph 26).

The present invention still further relates to: a method for screening an inhibitor or a promoter of effects of controlling cell migration and/or cell death, wherein the protein according to any one of paragraphs 10 to 16, the peptide according to paragraph 17 or 18, or a cell membrane expressing the protein according to any one of paragraphs 10 to 16 or the peptide according to paragraph 17 or 18, and a test substance are used (paragraph 27); a method for screening an inhibitor or a promoter of effects of controlling cell migration and/or cell death, or an inhibitor or a promoter of the expression of the protein according to any one of paragraphs 10 to 16 or of the peptide according to paragraph 17 or 18, wherein a cell expressing the protein according to any one of paragraphs 10 to 16 or the peptide according to paragraph 17 or 18, and a test substance are used (paragraph 28); and a method for screening an inhibitor or a promoter of effects of controlling cell migration and/or cell death, or an inhibitor or a promoter of the expression of the protein to any one of paragraphs 10 to 16 or the peptide according to paragraph 17 or 18, wherein the non-human animal according to any one of paragraphs 24 to 26 and a test

substance are used (paragraph 29).

The present invention also relates to: the promoter of effects of controlling cell migration and cell death obtained by the method for screening according to any one of paragraphs 27 to 29 (paragraph 30); the inhibitor of effects of controlling cell migration and cell death obtained by the method for screening according to any one of paragraphs 27 to 29 (paragraph 31); a promoter of the expression of the protein according to any one of paragraphs 10 to 16 or of the peptide according to paragraph 17 or 18, being obtained by the method for screening according to any one of paragraphs 27 to 29 (paragraph 32); an inhibitor of the expression of the protein according to any one of paragraphs 10 to 16 or of the peptide according to paragraph 17 or 18, being obtained by the method for screening according to any one of paragraphs 27 to 29 (paragraph 33); the inhibitor of metastasis of a cancer/a tumor, or a regulant of cell migration for transplantation treatment that includes the protein according to any one of paragraphs 10 to 16, the peptide according to paragraph 17 or 18, the recombinant protein or the recombinant peptide according to paragraph 22, the antibody according to paragraph 20 or 21, the inhibitor of effects of controlling cell migration and cell death according to paragraph 31, or the inhibitor of the expression according to paragraph 33 as an active ingredient (paragraph 34).

## BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Fig. 1 is a photograph showing the localization of L-FILIP cDNA or S-FILIP cDNA of the present invention and the structure of FILIPs.

Fig. 2 is a photograph showing the results as to the interaction of L-FILIP or S-FILIP of the present invention with Filamin 1 that is an actin-

binding protein.

Fig. 3 is a photograph showing the results of degradation of Filamin 1 by L-FILIP or S-FILIP of the present invention and reduction of cell motility thereby.

Fig. 4 is a photograph showing the results as to control of cell migration from ventricular zone by L-FILIP or S-FILIP in the formation of neocortex.

## BEST MODE FOR CARRYING OUT THE INVENTION

As a protein according to the present invention, a protein that comprises an amino-acid in which one or several amino acids are deleted, substituted or added, and has effects of controlling cell migration and cell death in L-FILIP shown in SEQ ID NO: 2, S-FILIP shown in SEQ ID NO: 4, or an amino-acid sequence shown in SEQ ID NOS: 2 or 4 is exemplified. Aforementioned effects of controlling cell migration and cell death are effects of controlling cell motility and cell death. The protein can be prepared by known methods on the basis of its DNA sequence information etc., and its derivation is not limited specifically. A Peptide being an object of the present invention is not particularly limited as long as it is a peptide that consists of a part of a protein of the present invention and has effects of controlling cell migration and cell death. Aforementioned protein and peptide being an object of the present invention, and recombinant protein and peptide to which the antibody, which specifically binds to these proteins and peptides, specifically binds, may be generically called hereinafter as “the proteins/peptides of the present invention”. Meanwhile, the proteins/peptides of the present invention can be prepared by known method on the basis of its DNA sequence information etc., and its derivation is not particularly limited to rat.

An antibody that "specifically binds" to a protein is one that binds to a protein, but which does not recognize and bind to other molecules in a sample, e.g., a biological sample, which naturally includes the protein.

As a DNA being an object of the present invention, any DNA can be used as long as it encodes the aforementioned protein of the present invention, for instance, a DNA encoding L-FILIP shown in SEQ ID NO: 2 in the sequence listing, a DNA encoding S-FILIP shown in SEQ ID NO: 4 in the sequence listing and a protein comprising an amino-acid in which one or several amino acids are deleted, substituted or added in SEQ ID NOS: 2 or 4 in the sequence listing, and having effects of controlling cell migration and cell death or an amino-acid sequence shown or a DNA comprising base sequence shown in SEQ ID NOS: 1 or 3 in the sequence listing, its complementary sequence, and part or whole of these sequences, are exemplified specifically. These can be prepared by known method on the basis of its DNA sequence information etc., for example, from gene library or cDNA library of human, mouse, rat, rabbit, and the like.

Also contemplated by the present invention are proteins or peptides which are at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% homologous to any of SEQ ID NOS: 2, 4, and 6.

A DNA that encodes the novel protein comprising effects of controlling cell migration and cell death can also be obtained by performing hybridization with the DNA comprising base sequence shown in SEQ ID NOS: 1 or 3 in the sequence listing, its complemented sequence, and part the whole of these sequences as a probe in stringent condition to various DNA libraries and isolating the DNA hybridizing with the probe. The DNA obtained in this manner is also within the scope of the present invention. As a hybridization condition for obtaining the DNA of the present invention, hybridization at 42°C and rinse at 42°C with buffer including 1 × SSC and 0.1% SDS, more preferably hybridization at 65°C and rinse at 65°C with buffer including 0.1 × SSC and 0.1% SDS is exemplified. Although, factors that influence on the

stringency of hybridization include various factors other than above-mentioned temperature condition, those skills in the art can achieve stringency equivalent to the stringency of above-mentioned hybridization by combining various factors properly.

Also contemplated by the present invention are DNA sequences or fragments which are at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% homologous to any of SEQ ID NOS: 1, 3, or 5.

Sequence identity with respect to any of the sequences presented here can be determined by a simple "eyeball" comparison (i.e. a strict comparison) of any one or more of the sequences with another sequence to see if that other sequence has, for example, at least 70% sequence identity to the sequence(s).

Alternatively, relative sequence identity can also be determined by commercially available computer programs that can calculate % identity between two or more sequences using any suitable algorithm for determining identity, using for example default parameters. A typical example of such a computer program is CLUSTAL. Other computer program methods to determine identify and similarity between the two sequences include but are not limited to the GCG program package (Devereux *et al* 1984 Nucleic Acids Research 12: 387) and FASTA (Atschul *et al* 1990 J Molec Biol 403-410).

The sequence identity or percent homology for proteins and nucleic acids can also be calculated as $(N_{ref} - N_{dif}) \times 100/N_{ref}$, wherein $N_{dif}$ is the total number of non-identical residues in the two sequences when aligned and wherein $N_{ref}$ is the number of residues in one of the sequences. Hence, the DNA sequence AGTCAGTC will have a sequence identity of 75% with the sequence AATCAATC ($N_{dif}$=2 and $N_{ref}$=8).

Percent homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one

sequence is directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause the following amino acid residues to be put out of alignment, thus potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalising unduly the overall homology score. This is achieved by inserting "gaps" in the sequence alignment to try to maximise local homology.

However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible - reflecting higher relatedness between the two compared sequences - will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example, when using the GCG Wisconsin Bestfit package the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum % homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (University of Wisconsin, U.S.A.; Devereux *et al.*,

1984, Nucleic Acids Research 12:387). Examples of other software than can perform sequence comparisons include, but are not limited to, the BLAST™ package (Ausubel *et al.*, 1999 *ibid* – Chapter 18), FASTA (Atschul *et al.*, 1990, J. Mol. Biol., 403-410) and the GENEWORKS™ suite of comparison tools. Both BLAST™ and FASTA are available for offline and online searching (Ausubel *et al.*, 1999 *ibid*, pages 7-58 to 7-60).

Although the final % homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix - the default matrix for the BLAST™suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied. It is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

Advantageously, the BLAST™ algorithm is employed, with parameters set to default values. The BLAST™ algorithm is described in detail at www.ncbi.nih.gov/BLAST/blast_help.html, which is incorporated herein by reference. The search parameters are defined as follows, can be advantageously set to the defined default parameters.

Advantageously, "substantial identity" when assessed by BLAST™ equates to sequences which match with an EXPECT value of at least about 7, preferably at least about 9 and most preferably 10 or more. The default threshold for EXPECT in BLAST™ searching is usually 10.

BLAST™ (Basic Local Alignment Search Tool) is the heuristic search algorithm employed by the programs blastp, blastn, blastx, tblastn, and tblastx; these programs ascribe significance to their findings using the statistical methods of Karlin and Altschul (Karlin and Altschul 1990, *Proc. Natl. Acad. Sci. USA* 87:2264-68; Karlin and Altschul, 1993, *Proc. Natl. Acad. Sci. USA*

90:5873-7; see www.ncbi.nih.gov/BLAST/blast_help.html) with a few enhancements. The BLAST™programs are tailored for sequence similarity searching, for example to identify homologues to a query sequence. For a discussion of basic issues in similarity searching of sequence databases, see Altschul *et al* (1994) Nature Genetics 6:119-129.

The five BLAST™ programs available at www.ncbi.nlm.nih.gov perform the following tasks: **blastp** - compares an amino acid query sequence against a protein sequence database; **blastn** - compares a nucleotide query sequence against a nucleotide sequence database; **blastx** - compares the six-frame conceptual translation products of a nucleotide query sequence (both strands) against a protein sequence database; **tblastn** - compares a protein query sequence against a nucleotide sequence database dynamically translated in all six reading frames (both strands); **tblastx** - compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.

BLAST™ uses the following search parameters:

HISTOGRAM - Display a histogram of scores for each search; default is yes. (See parameter H in the BLAST™ Manual).

DESCRIPTIONS - Restricts the number of short descriptions of matching sequences reported to the number specified; default limit is 100 descriptions. (See parameter V in the manual page).

EXPECT - The statistical significance threshold for reporting matches against database sequences; the default value is 10, such that 10 matches are expected to be found merely by chance, according to the stochastic model of Karlin and Altschul (1990). If the statistical significance ascribed to a match is greater than the EXPECT threshold, the match will not be reported. Lower EXPECT thresholds are more stringent, leading to fewer chance matches being reported. Fractional values are acceptable. (See parameter E in the BLAST™ Manual).

CUTOFF - Cutoff score for reporting high-scoring segment pairs. The

default value is calculated from the EXPECT value (see above). HSPs are reported for a database sequence only if the statistical significance ascribed to them is at least as high as would be ascribed to a lone HSP having a score equal to the CUTOFF value. Higher CUTOFF values are more stringent, leading to fewer chance matches being reported. (See parameter S in the BLAST™ Manual). Typically, significance thresholds can be more intuitively managed using EXPECT.

ALIGNMENTS - Restricts database sequences to the number specified for which high-scoring segment pairs (HSPs) are reported; the default limit is 50. If more database sequences than this happen to satisfy the statistical significance threshold for reporting (see EXPECT and CUTOFF below), only the matches ascribed the greatest statistical significance are reported. (See parameter B in the BLAST™ Manual).

MATRIX - Specify an alternate scoring matrix for BLASTP, BLASTX, TBLASTN and TBLASTX. The default matrix is BLOSUM62 (Henikoff & Henikoff, 1992). The valid alternative choices include: PAM40, PAM120, PAM250 and IDENTITY. No alternate scoring matrices are available for BLASTN; specifying the MATRIX directive in BLASTN requests returns an error response.

STRAND - Restrict a TBLASTN search to just the top or bottom strand of the database sequences; or restrict a BLASTN, BLASTX or TBLASTX search to just reading frames on the top or bottom strand of the query sequence.

FILTER - Mask off segments of the query sequence that have low compositional complexity, as determined by the SEG program of Wootton & Federhen (1993) Computers and Chemistry 17:149-163, or segments consisting of short-periodicity internal repeats, as determined by the XN U program of Claverie & States (1993) Computers and Chemistry 17:191-201, or, for BLASTN, by the DUST program of Tatusov and Lipman (see www.ncbi.nlm.nih.gov). Filtering can eliminate statistically significant but biologically uninteresting reports from the BLAST™ output (e.g., hits against

common acidic-, basic- or proline-rich regions), leaving the more biologically interesting regions of the query sequence available for specific matching against database sequences.

Low complexity sequence found by a filter program is substituted using the letter "N " in nucleotide sequence (e.g., "NNNNNNNNNNNNN") and the letter "X" in protein sequences (e.g., " XXXXXXXXX").

Filtering is only applied to the query sequence (or its translation products), not to database sequences. Default filtering is DUST for BLASTN, SEG for other programs.

It is not unusual for nothing at all to be masked by SEG, XNU, or both, when applied to sequences in SWISS-PROT, so filtering should not be expected to always yield an effect. Furthermore, in some cases, sequences are masked in their entirety, indicating that the statistical significance of any matches reported against the unfiltered query sequence should be suspect.

NCBI-gi - Causes NCBI gi identifiers to be shown in the output, in addition to the accession and/or locus name.

Most preferably, sequence comparisons are conducted using the simple BLAST™ search algorithm provided at www.ncbi.nlm.nih.gov/BLAST. In some embodiments of the present invention, no gap penalties are used when determining sequence identity.

As a fusion protein and a fusion peptide of the present invention, any fusion protein and fusion peptide can be used as long as the proteins/peptides of the present invention is bound to marker protein and/or peptide tag. A marker protein is not particularly limited as long as it is a marker protein conventionally known, alkaline phosphatase, Fc region of antibodies, HRP, GFP, and the like are exemplified as specific examples. Further, as a peptide tag of the present invention, a peptide tag conventionally known such as HA tag, Myc tag, His tag, FLAG™ tag, and GST tag are specifically exemplified. The fusion protein can be prepared by known methods. It is useful in purification of the protein and the like having effects of controlling cell

migration and cell death by utilizing affinity of Ni-NTA and His tag, in detection of protein having effects of controlling cell migration and cell death, and in quantification of antibodies directed against a protein having effects of controlling cell migration and cell death. It is also useful as an inhibiror of cancer and tumor metastasis or a regulant of cell migration for transplantation therapy, and a reagent for research in the field concerned.

As an antibody that binds specifically to the proteins or peptides of the present invention, an immunity-specific antibody such as monoclonal antibody, polyclonal antibody, chimeric antibody, single-stranded antibody, and humanized antibody are exemplified as specific examples, where these antibodies can be prepared by known methods using the whole or a part of the above-mentioned proteins/peptides of the present invention, fusion protein, fusion peptide, and the like as an antigen, and monoclonal antibody is more preferable among them in view of specificity. The antibody such as monoclonal antibody is useful, for instance not only as an inhibitor of cancer and tumor metastasis or a regulant of cell migration for transplantation therapy, but also in clarifying the mechanism of such as cancer and tumor metastasis and cell migration of neuron and the like.

The aforementioned antibody of the present invention is produced by administering the proteins/peptides of the present invention, a fragment thereof containing epitope, or a cell expressing the protein on the surface of its membrane to an animal (preferably other than human) with the use of conventional protocol, for example, the monoclonal antibody can be prepared by an arbitary method which brings antibodies developed by cultured materials of continuous cell line, such as hybridoma method (Nature 256, 495-497, 1975), trioma method, human B cell hybridoma method (Immunology Today 4, 72, 1983), and EBV-hybridoma method (MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985) can be used.

A preparation method of single-stranded antibody (USSN 4,946,778) can be applied in order to the preparation of single-stranded antibody against

the above-mentioned proteins/peptides of the present invention. Further, a humanized antibody can be expressed by using a transgenic mouse or other mammal, a clone expressing the proteins/peptides of the present invention can be isolated/identified with the above-mentioned antibody, or the polypeptide can be purified with affinity chromatography. The antibodies against the proteins/peptides of the present invention may be used usefully as an inhibitor of cancer and tumor metastasis or a regulant of cell migration for transplantation therapy, and may also be used usefully in clarifying the mechanism of such as cancer and tumor metastasis and cell migration of neuron and the like, as aforementioned. Further, the recombinant protein or peptide to which these antibodies specifically bind are also included in the proteins/peptides of the present invention as aforementioned.

Functions of the proteins/peptides of the present invention can be analyzed by using, for example, antibodies such as the aforementioned monoclonal antibodies and the like that are labeled with fluorescent materials such as FITC (Fluorescein isothiocyanate), tetramethylrhodamine isothiocyanate, etc., radioisotopes such as $^{125}I$, $^{32}P$, $^{14}C$, $^{35}S$, $^{3}H$, etc. or enzymes such as alkaline phosphatase, peroxidase, β-galactosidase, phycoerythrin, etc. and by using fusion proteins fused with fluorescence proteins such as Green Fluorescent Protein (GFP) etc. As for immunological detection methods using the antibodies of the present invention, RIA method, ELISA method, fluorescent-antibody method, plaque method, spot method, hemagglutination, Ouchterlony method, etc. are exemplified.

The present invention also relates to a host cell comprising an expression system, which is able to express the proteins/peptides of the present invention. Introduction of a gene that encodes the proteins/peptides of the present invention into a host cell can be performed by the method written in a number of standard laboratory manuals such as of Davis et al. (BASIC METHODS IN MOLECULAR BIOLOGY, 1986), and of Sambrook et al. (MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed., Cold Spring

Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), for example, calcium phosphate transfection, DEAE-dextran-mediated transfection, transvection, microinjection, cationic lipid-mediated transfention, electroporation, transduction, scrape loading, ballistic introduction, and infection. As a host cell; bacterial procaryotic cell, such as Escherichia coli, Streptomyces, Bucillus subtilis, Streptococcus, and Staphylococcus; fungal cell, such as yeast and Aspergillus; insect cell, such as Drosophila S2 and Spodoptera Sf9; animal cell, such as L cell, CHO cell, COS cell, NIH3T3 cell, HeLa cell, C127 cell, BALB/c3T3 cell (including a mutant strain deficient in dihydrofolate reductase or thymidine kinase), BHK21 cell, HEK293 cell, and Bowes malignant melanoma cell; and plant cell can be exemplified.

As an expression system, any expression system can be used as long as it is an expression system that can express the proteins/peptides of the present invention in a host cell, an expression system derived from chromosome, episome, mammal, or virus, for example; a vector derived from bacterial plasmid, yeast plasmid, papova virus such as SV40, vaccinia virus, adenoviirus, fowl poxvirus, pseudorabies virus, retrovirus; a vector derived from bacteriophage, transposon, or combination of these vectors, for example a vector derived from genetic component of plasmid and bacteriophage such as cosmid or phagemid can be exemplified as a specific examples. The expression system may not only yield expression but also include a regulatory sequence for controlling expression.

A host cell comprising the aforementioned expression system, a cell membrane of the cell, and the proteins/peptides of the present invention obtained by culture of the cell can be used for a method for screening the present invention as described below. For example, as the method for obtaining cell membrane, the method by F. Pietri-Rouxel et al. (Eur. J. Biochem., 247, 1174-1179, 1997) and the like can be used. For recovering and purifying the proteins/peptides of the present invention from cell culture, the known methods including ammonium sulfate - or ethanol precipitation, acid extraction, anion or

cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxyapatite chromatography, and lectin chromatography, preferably, high-performance liquid chromatography is used. As a column especially used for affinity chromatography, columns to which antibodies to the proteins/peptides of the present invention are bound, for instance, are used and when ordinary peptide tags are added to the proteins/peptides of the present invention mentioned above, columns to which substances having affinity with the peptide tags are bound are used in order to obtain the proteins/peptides of the present invention. The method for purifying the proteins/peptides of the present invention can also be applied for peptide synthesis.

In the present invention, a non-human animal whose gene function to encode the proteins/peptides of the present invention mentioned above is deficient on its chromosome means a non-human animal part or whole of whose gene on its chromosome encoding the proteins/peptides of the present invention is inactivated by gene mutation such as destruction, deletion, substitution, etc. do that whose function to express the proteins/peptides the present invention is lost. Further, a non-human animal which over-expresses the proteins/peptides of the present invention is specifically exemplified by a non-human animal which produces larger amount of the proteins/peptides of the present invention than a wild-type non-human animal does. Although rodents or the like such as mice, rats, etc. are particularly exemplified for non-human animals of the present invention, the examples will not be limited to these animals only.

Homozygous non-human animals that are born according to Mendel's Law include a deficient type or the over-expressing type for the proteins/peptides of the present invention, as well as their wild-type littermates. By using the deficient type animals or the over-expressing type animals of these homozygous non-human animals together with their wild-type littermate at the same time, accurate comparative experiments can be carried out on the individual level. In performing screening of the present invention described

below, it is, therefore preferable to use the wild type non-human animals, i.e. animals of the same species as or even better the littermates of, non-human animals whose gene function to encode the proteins/peptides of the present invention is deficient or over-expressing on their chromosomes with a combination of the deficient or over-expressing type animals. The method of producing a non-human animal whose function to encode the proteins/peptides of the present invention is deficient or over-expressing on its chromosome is now explained in the following with an L-FILIP knockout mouse and an L-FILIP transgenic mouse as examples.

A mouse, for instance, whose gene function to encode the L-FILIP protein is deficient on its chromosome, i.e. an L-FILIP knockout mouse is generated by the following steps. A gene encoding mouse L-FILIP, which is homologous to rat L-FILIP, is screened by using a gene fragment obtained by a method such as PCR or the like from the mouse gene library. A screened gene which encodes mouse L-FILIP is subcloned with a viral vector or the like and is then identified by DNA sequencing. The whole or part of a gene of this clone which encodes mouse L-FILIP is substituted with a pMC1 neo gene cassette or the like. A gene such as a diphtheria toxin A fragment (DT-A) gene, a herpes simplex virus thymidine kinase (HSV-tk) gene, etc. is introduced onto the 3'-end, and thus a targeting vector is constructed.

The targeting vectors thus constructed are linearized and introduced into ES cells by electroporation or the like to cause homologous recombination. Among the homologous recombinants, ES cells in which homologous recombination have occurred are selected by the use of antibiotics such as G418, ganciclovir (GANC), etc. It is preferable to confirm whether the ES cells selected are the recombinants of the interest by Southern blotting or the like. A clone of the ES cells confirmed is microinjected into a mouse blastocyst, and which blastocyst is placed back to the recipient mouse to generate a chimeric mouse. A heterozygous mouse can be obtained by intercrossing the chimeric mouse with a wild-type mouse. By further

intercrossing the heterozygous mice, the L-FILIP knockout mice of the present invention can be generated. Whether the ability of expressing L-FILIP is lost in an L-FILIP knockout mouse is examined by Northern blotting upon isolating RNA from the mouse obtained by the above-described method and Western blotting or the like with which the L-FILIP expression in the mouse can be directly examined.

An L-FILIP transgenic mouse is created by following steps. A promoter such as chicken β-actin, mouse neurofilament, SV40, etc. and poly (A) such as rabbit β-globin SV40, etc. or introns are fused with cDNA encoding L-FILIP derived from human, mouse, rat, rabbit, etc., to construct a transgene. This transgene is microinjected into the pronuclear of mouse fertilized egg. After the obtained egg cell is cultured, it is transplanted to the oviduct of the recipient mouse which was fed thereafter. Neonatal mice that have the aforementioned cDNA were selected from among all the mice born and thus the transgenic mice are created. Neonatal mice having the cDNA can be selected by extracting crude DNA from the mice tails or the like and then by a dot hybridization method using a gene encoding the introduced L-FILIP as a probe and by PCR method or the like using a specific primer.

The host cell that comprises: the gene or DNA which encodes the above-mentioned proteins/peptides of the present invention; the proteins/peptides of the present invention; the fusion protein of the proteins/peptides of the present invention combined with marker protein and/or peptide tag; antibodies against the proteins/peptides of the present invention; and an expression system which is able to express the proteins/peptide of the present invention is useful for an inhibitor for metastasis of a cancer or a tumor or a regulant of cell migration for transplantation treatment as described below specifically. It is also available for elucidation of mechanisms such as metastasis of a cancer or a tumor or cell migration of neuron and the like as well as control of cell migration and/or cell death, a method for screening an inhibitor or a promoter of effects of controlling cell migration and/or cell death,

or a method for screening an inhibitor or a promoter for expressing the proteins/peptides of the present invention.

As the method for screening an inhibitor or a promoter for controlling cell migration and/or cell death of the present invention: a method using the above-mentioned proteins/ peptides of the present invention or a cell membrane expressing the proteins/peptides of the present invention, and a test substance, a method using a cell membrane expressing the above-mentioned proteins/peptides of the present invention and a test substance, a method using non-human animal such as knockout mouse or transgenic mouse of the proteins/peptides of the present invention and a test substance, and others are exemplified. Further, a method using a cell membrane expressing the above-mentioned proteins/peptides of the present invention and a test substance, a method using non-human animal such as knockout mouse or transgenic mouse of the proteins/peptides of the present invention and a test substance, and others can be used for a method for screening an inhibitor or a promoter for expressing the proteins/peptides of the present invention.

As the method for screening that uses the above-mentioned the proteins/peptides of the present invention or a cell membrane expressing the proteins/peptides of the present invention and a test substance, a method of measuring and evaluating effects of controlling cell migration and cell death of the proteins/peptides of the present invention, by contacting the proteins/peptides or the proteins/peptides expressing on the surface of cell membrane with a test substance can be specifically exemplified. As the method for screening that uses a cell expressing the proteins/peptides of the present invention and a test substance, a method of measuring and evaluating effects of controlling cell migration and cell death of the proteins/peptides of the present invention, or the variation of expression amounts of the proteins/peptides of the present invention, by contacting a cell expressing the proteins/peptides of the present invention with a test substance can be specifically exemplified.

As the method for screening that uses non-human animal whose

function of gene for encoding above-mentioned protein and peptide of the present invention is deficient on the chromosome or non-human animal which over-expresses the proteins/peptides of the present invention and a test substance, a method for measuring and evaluating the effects of controlling cell migration and cell death of the proteins/peptides of the present invention or the variation in expression amounts of the proteins/peptides of the present invention by contacting the cell or the tissue obtained from these non-human animal with a test substance in vitro, a method for measuring and evaluating the effects of controlling the cell migration and cell death of the proteins/peptides of the present invention or the variation in expression amounts of the proteins/peptides of the present invention at the cell or the tissue obtained from these non-human animal after administering a test substance in advance to the non-human animal whose function of gene for encoding the proteins/peptides of the present invention is deficient on the chromosome or non-human animal which over-expresses the proteins/peptides of the present invention, a method for measuring and evaluating effects of controlling cell migration and cell death of the proteins/peptides of the present invention or the variation in expression amounts of the proteins/peptides of the present invention at the non-human animal after administering a test substance in advance to the non-human animal whose function of gene for encoding the proteins/peptides of the present invention is deficient on the chromosome or non-human animal which over-expresses the proteins/peptides of the present invention, and etc., are specifically exemplified.

The promoter of effects of controlling cell migration and cell death or the promoter of expression of the present invention obtained from the screening method mentioned above can be used for such as treatment of patients requiring promotion of effects of controlling cell migration and cell death, or promotion of expression of the proteins/peptides of the present invention. The inhibitor of effects of controlling cell migration and cell death or the expression inhibitor of the present invention obtained from the screening method mentioned above can

be used for such as treatment of patients requiring inhibition of effects of controlling cell migration and cell death, or inhibition of expression of the proteins/peptides of the present invention. The proteins/peptides of the present invention or the antibody against it can be used as an active ingredient for an inhibitor of cancer and tumor metastasis or a regulant of cell migration for transplantation therapy, and the like. It can be used missile therapy, as well.

The active ingredient(s) of a pharmaceutical composition is contemplated to exhibit excellent therapeutic activity, for example, in the treatment of cancer. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. Dosages may be administered at intervals of the course of several days, weeks, months or years.

The active compound may be administered in a convenient manner such as by the oral, intravenous (where water soluble), intramuscular, subcutaneous, intranasal, intradermal or suppository routes or implanting (e.g. using slow release molecules). The method of administration may depend on factors such as the location of the cancer or other ailment in the body which is to be treated. Depending on the route of administration, the active ingredient may be required to be coated in a material to protect said ingredients from the action of enzymes, acids and other natural conditions which may inactivate said ingredient.

In order to administer the combination by other than parenteral administration, it will be coated by, or administered with, a material to prevent its inactivation. For example, the combination may be administered in an adjuvant, co-administered with enzyme inhibitors or in liposomes. Adjuvant is used in its broadest sense and includes any immune stimulating compound such as interferon. Adjuvants contemplated herein include resorcinols, non-ionic surfactants such as polyoxyethylene oleyl ether and n-hexadecyl polyethylene ether. Enzyme inhibitors include pancreatic trypsin.

Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes.

The active compound may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene gloycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of superfactants.

The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thirmerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered

sterilisation. Generally, dispersions are prepared by incorporating the sterilised active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the combination of polypeptides is suitably protected as described above, it may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsules, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained.

The tablets, troches, pills, capsules and the like may also contain the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier.

Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should

be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

As used herein "pharmaceutically acceptable carrier and/or diluent" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such as active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired.

Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985).

The principal active ingredients are compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

For example, in one aspect these therapeutic agents can be administered orally or parenterally. Pharmaceutical solid such as powders, granule, capsules, and tablets or pharmaceutical liquid such as syrup or elixir can be an oral administration agent, or can be an injection, a percutaneous preparation, suppository, or the like as a parenteral administration agent. These formulations can be produced in a usual manner by adding an auxiliary agent admitted pharmacologically and pharmaceutical technologically to active constituents. For example, components of formulation of diluents such as light silicic acid anhydride, starch, lactose, crystalline cellulose, and lactose calcium, disintegrator such as carboxy methyl cellulose and of lubricant such as magnesium stearate for oral agent and muscal administration agent, components of formulation of solubilizer or auxiliary solubilizer such as saline, mannitol, and propylene glycol, and of suspention such as surface active agent for injection, and further components of formulation of solubilizer or auxiliary solubilizer of water or oil-based and of adhesive for external preparation are used as an auxiliary agent.

Applied dose can be determined properly depending on the kind of objective disease, age, gender, body weight, and symptom of patient and administration pattern.

Depending upon the need, the complex(es) may be administered at a dose of from about 0.001 to about 30 mg/kg body weight, such as from about 0.1 to about 10 mg/kg, or from about 0.1 to about 1 mg/kg body weight.

Of course, for any composition to be administered to an animal or human, and for any particular method of administration, it is preferred to determine therefore: toxicity, such as by determining the lethal dose (LD) and $LD_{50}$ in a suitable animal model e.g., rodent such as mouse; and, the dosage of the composition(s), concentration of components therein and timing of administering the composition(s), which elicit a suitable response. Such determinations do not require undue experimentation from the knowledge of the skilled artisan, this disclosure and the documents cited herein.

The present invention will be explained more specifically with examples below, but the scope of the present invention is not limited to these examples. In the following example, Wister rats (Keari; SLC) which were housed with food and water ad libitum under a constant temperature and humidity were used. For the animal mentioned above, embryonic day 0 (E0) is defined as the day of confirmation of the vaginal plug and day of birth is designated as P0 (postnatal day 0). Rats of P0 to P7 were anesthetized by hypothermia, while Rats of P14 to adults including being pregnant were anesthetized by intraperitoneal injection of sodium pentobarbital at 40 mg/kg.

Example 1 (Isolation of FILIP cDNA and localization of FILIP)

Although it has been known that Filamin 1 (ABP-280), an actin binding protein, is an essential component of the radial migratory machinery for obtaining postmitotic neocortical neurons (Neuron 21, 1315-1325, 1998), its expression in migratory and postmigratory neurons involved in the development of neocortex, in the region from the intermediate zone to the cortical plate (Neuron 21, 1315-1325, 1998), suggests that the start of migration out of the ventricular zone is possibly controlled by other system. In order to elucidate the molecules concerning controlling the start of neuronal migration, mRNA differential display, in situ hybridization histochemistry, and screening of rat cDNA library were conducted according to the method written in Mol. Brain. Res. 62, 187-195, 1998. First, genes that expressed more abundantly in the neocortics of Wistar rats on embryonic day 11 to 12 (E11 to 12) compared with Wistar rats on embryonic day 18 to 20 (E18 to 20) were isolated by mRNA differential display. Postmitotic neurons were at the stage of migrating out of the ventricular zone toward the pial surface on E12 Wistar rats, whereas most of them had already left there at around E18 to 20 by which neurogenesis was complete. The two hundred gene fragments obtained from the results above, which expressed dramatically on E12 but not so much on E18 to 20 were sequenced and 80 independent clones were obtained by excluding overlaps.

Then, further selection was performed by in situ hybridization histochemistry with a part of the full-length of rat S-FILIP (165 nucleotides; base sequence 1289-1453) as a probe. Consequently, among 80 independent clones, one novel clone showing expression in the ventricular zone of the cortex was isolated, which was named filip (Filamin-interacting protein). In order to investigate expression of the FILIP (S-FILIP) gene, in situ hybridization for rats E12 and 18 was performed with its sagittal section. The results were shown in Fig. 1a. According to this, positive signal was confirmed at ventricular zone of cortex (cx) and superior colliculus (sc) in central nervous system of E12 (Fig. 1a left). The signal could not been confirmed abundantly at ventricular zone of E18, however, they were confirmed abundantly at heart, aorta, gastrointestinal tract, and diaphragm, and filip gene was found that they expressed at myocardium, skeletal muscle, and smooth muscle (Fig. 1a right). The scale bar in Fig. 1a shows 1 mm.

cDNA library derived from frontal cortex of a Wistar rat E11 was constructed and screened FILIP with the probe used at above-mentioned in situ hybridization selection and a MARATHON™ cDNA Amplification Kit (CLONTECH). Genetic information from the DNA database of Japan (DDBJ) was utilized in part to isolate FILIP cDNA. Consequently, two full-length FILIP cDNAs, different only in their 5'termini, with regions recognizing the aforementioned probes were obtained. Amino acid sequences were determined from the information of the two cDNA information respectively. The structure is shown in Fig. 1b. As a result, structures were confirmed to coincide with each other except S-FILIP (short form FILIP; GENBANK™ accession number D87257) lacked 247 residues at N-terminus of L-FILIP (long form FILIP; GENBANK™ accession number AB055759). The above-mentioned two proteins were confirmed to be intracellular proteins, since neither a signal sequence nor a transmembrane region was found by their hydrophobicity profiles. Though four leucine zipper motifs and coiled-coil region could be recognized in the C-terminal halves of S-FILIP (Fig. 1c), the amino acid

sequence of the regions were found that they did not show similarity to any protein which had been reported so far (Fig. 1b, c).

Next, in order to investigate the cellular localization of S-FILIP (fiber-like; Fig. 1d top) and L-FILIP (punctate; Fig. 1d bottom), mammalian expression vectors including FILIPs-GFP (pEGFP-N1 (CLONTECH), pCAGGS (Invitrogen) or pBudCE4 (Invitrogen)) which was tagged with green fluorescent protein (GFP) at C-termini of FILIP were transfected to COS-7 cells which were maintained in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) at 37°C in 5% $CO_2$, FILIPs-GFP was expressed, and image analysis was performed by OLYMPUS™ IX-70 micro scope equipped with a digital cooled CCD camera (Hamamatsu Photonics) (GFP in Fig. 1d). Further, in order to investigate whether above-mentioned FILIPs were coexistent with F-actin, after aforementioned COS-7 cells were fixed with 4% praformaldehyde/0.1 M phosphate buffer (PB) (pH 7.4) for 10 min, and permeabilized in 0.1% TRITON® X-100/phosphate buffered saline (PBS) for 3 min, F-actin was stained with rhodamine-phalloidin (1:40; Molecular Probes) (phalloidin in Fig. 1d), coexistence of S-FILIP or L-FILIP with F-actin was investigated (merged in Fig.1d). The results are shown in Fig. 1d. The scale bar in Fig. 1d indicates 10 μm. According to this result, S-FILIP tagged with GFP was localized along actin stress filaments generally except at their ends, and that the possibility of colocalization of S-FILIP with F-actin was presumed. In contrast, L-FILIP exhibited a punctate distribution in cytoplasm, unlike the colocalization of F-actin.

Meanwhile, 50 cells expressing S-FILIP tagged with above-mentioned GFP (Fig. 1e) or L-FILIP tagged with above-mentioned GFP (Fig. 1f) were extracted in a random order respectively, and the numbers of cells in each expression distribution pattern of FILIPs-GFP were measured. The measurement was performed four times, and the obtained results were calculated as the mean value ± S.E. M. The result showed that each pattern of colocalization was heavily dependent on the type of FILIP molecule. Then,

coexistence with F-actin in the region not including known actin-binding domain (N-terminus at S-FILIP) was investigated, as well. Expressions of S-FILIPΔC-GFP (C-terminus-deficient S-FILIP tagged with GFP), FILIPΔN (N-terminus-deficient FILIP tagged with GFP), or only GFP at COS-7 cell in the above-mentioned manner (Fig. 1g) suggested that S-FILIP coexist with F-actin (Fig. 1 center) despite the lack of existence of known actin-binding domain. This led to reveal that C-terminal halves (FILIPΔN) being common to S-FILIP and L-FILIP was essential and sufficient for colocalization with F-actin. In contrast, L-FILIP showed little colocalization with F-actin, however, it exhibited a punctate distribution in cytoplasm of the most cells. Further, actin stress filaments were scarcely observed in COS-7 cells which express L-FILIP. The scale bar in Fig. 1g indicates 20 μm.

Example 2 (Interaction of FILIPs with actin binding protein Filamin 1)

In order to further examine the unique localization of S-FILIP associated with F-actin, and elucidate the factor that might serve as a link between both molecules, a yeast two-hybrid screen was performed using the C-terminal half of S-FILIP (bait) and the whole embryo library (prey) of mouse E11. Using MATCHMAKER$^{TM}$ Two-Hybrid system (CLONTECH) for a yeast two-hybrid screen, the whole embryo library derived from brain of E11 mouse preintegrated into MATCHMAKER$^{TM}$ library (CLONTECH) was transformed with yeast strain PJ69-2A which was transformed with pAS2-1 plasmid vector carrying cDNA that encoded the common C-terminal region of FILIPs (residues 508-965 of the deduced amino acid sequence of S-FILIP), and C-terminal half of S-FILIP were mated with the whole embryo library of E11 mouse. As a result, over $8\times10^6$ clones were screened and 17 clones were selected based on three selection markers. In that way, a clone encoding Filamin 1, a protein interacting with actin filament, that interacts with F-actin into isotropic, orthogonal arrays and increases the viscosity and stiffness of the F-actin network was identified from these clones.

Next, L-FILIP-GFP, S-FILIP-GFP, a fusion protein where GFP bound to N-terminal half of S-FILIP tagged with GFP (S-FILIPΔC-GFP), a fusion protein where GFP bound to C-terminal half common to FILIPs (FILIPΔN-GFP), or cell lysates obtained from COS-7 cells expressing only GFP (protein solution solubilized in a buffer containing 20 mM TRIS™, (pH 7.5), 150 mM NaCl, 1000 U/ml DNase I, 1% NP-40, 1 mM phenylmethanesulfonyl fluoride, 5 μg/ml aprotinin, 1.5 μM pepstatin A, 2 μM leupeptin) were immnoprecipitated using either anti-GFP antibodies (CLONTECH) or anti-filamin 1 antibodies (Chemicon), and immunoprecipitated protein was detected with anti-Filamin 1 or anti-GFP antibodies as probes. The results of immunoprecipitation with anti-GFP antibodies are shown in Fig. 2a, and the results of immunoprecipitation with anti-Filamin 1 antibodies are shown in Fig. 2b. These results led to confirm the formation of complex comprising either full-length FILIPs or S-FILIP having C-terminal half and Filamin 1.

Further, in order to perform immunocytochemistry, or examine colocalization of S-FILIP (fiber-like; Fig. 2c) or L-FILIP (punctate; Fig. 2c) with Filamin 1, S-FILIP-GFP or L-FILIP-GFP was transfected with COS-7 cell, the cells were fixed to be permeabilized, and image analysis was performed with an OLYMPUS™ IX-70 microscope equipped with a digital cooled CCD camera (Hamamatsu Photonics) in following manner as mentioned in Example 1. After above-mentioned cells were permeabilized, the expression of endogenous Filamin 1 was blocked in 10% goat serum/PBS for 20 min, incubated in the coexistence of anti-Filamin 1 antibodies (1:200; Chemicon), then incubated and stained in the coexistence of anti-mouse Ig-Cy3 (1:400; Amersham-Pharmacia). These results are shown in Fig. 2c. Arrows in the figure indicate signals of FILIPs-GFP and Filamin 1 colocalizing interactively. The scale bars in upper and middle columns of the figure indicate 10 μm, and the scale bar in the lower column of the figure indicates 3 μm. As a result, it was confirmed that although not all but most of Filamin 1 were coexisted with S-FILIP signals (Fig.2c). In cells expressing L-FILIP, about half of the

punctate signals were confirmed to colocalized with Filamin 1 punctate signals. Accordingly, the present inventors designated these novel molecules Filamin 1-inteacting proteins, FILIPs.

Example 3 (Degradation of Filamin 1 by FILIPs and decrease of cell motility by the degradation)

Since Filamin 1 is deeply involved in cell migration in various cells (Science 255, 325-327, 1992), it is probable that FILIPs control cell migration via Filamin 1. Thus, investigation was performed whether or not FILIPs affect cell migration rate by introducing FILIPs into COS-7 cells, which possessed Filamin 1 but not FILIPs. On the day after plating (approximately $1 \times 10^4$ cells per 1.88 $cm^2$ area), for analyzing the ratio of cell migration rate, COS-7 cells were transfected with expression vectors including S-FILIP-GFP (Fig. 3a left), L-FILIP-GFP (Fig. 3a center), and GFP only (Fig. 3a right). After 36 to 48 h of transfection, the cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) on an IX-70 microscope equipped with n IX-IBC culturing apparatus (OLYMPUS™) under low cell density condition, image was analyzed twice at an interval of 120 min (Fig. 3a). [The images of GFP in Fig. 3a (green) were analyzed at an interval of 120 min, and then after the later ones had been converted to red color, these two images were merged.]. Further, in order to quantify cell migration in Fig. 3a, migrating distance (the mean value ± s. e. m.) of each of the nucleus (n=20 for S-FILIP-GFP, n=19 for L-FILIP-GFP, and n=18 for GFP alone) was measured by each group at an interval of 120 min (Fig.3b). The scale bar in Fig.3a shows 50 μm, in Fig. 3b, the cell migration occurred in the second image analysis was quantified by using phase-contrast image in combination. According to these results, it was confirmed that cell migration rate of the cells expressing FILIPs-GFP was reduced compared with the cells expressing GFP alone, under low cell density conditions, in which cell can migrate freely without interfering with other cells as well.

Next, in order to elucidate the effect of FILIPs on lamellipodium formation, a wound healing assay was performed. Over-confluent COS-7 cells were tranfected with the expression vectors including either FILIPs-GFP or GFP. After 36 to 48 h of transfention, defects were made among cells (Fig. 3b), and after they were cultured for further 3 h, they were fixed and stained with rhodamine-phalloidn. After the staining, the defect edges among the cells were observed to confirm whether they had lamellipodia. The results are shown in Fig. 3c. Arrows in the figure show the defect edges at the S- and L-FILIP-GFP expression cells, arrowheads show the neighboring cells that express no FILIPs respectively, the scale bar indicates 50 μm. Among the aforementioned COS-7 cells which formed defects, 50 cells were extracted at random, the cells where lamellipodium were formed at the wound edge and green GFP signals (FILIPs-GFP or GFP alone) were seen in the region were counted among them. As a result, it was confirmed that most of quiescent cells in the over-confluent state developed lamellipodia (sheet-like processes) in response to migration of neighboring cells. As shown in Fig. 3c, most cells expressing S- or L-FILIP-GFP did not form lamellipodia at the wound edge, compared with the cells that did not express FILIPs. In the cells expressing GFP (control), formation rate of lamellipodia was 68% at their wound edges, whereas formation rate of lamellipodia in the cells expressing S- and L-FILIP-GFP were only 28% and 4%, respectively. These results suggest that FILIPs suppress lamellipodium formation and cell migration, and lead to the consideration that FILIPs has inhibitory effects for the function of Filamin 1.

The molecular mechanism for the inhibitory effects of FILIPs on Filamin 1 was further examined by expressing recombinant FILIPs and recombinant Filamin 1 simultaneously in the same COS-7 cells using single expression vector including dual promoters. As shown in Fig. 3d, IRES (internal ribosomal entry site) sequence was inserted between HA-tagged Filamin 1 cDNA (HA-Filamin 1) and GFP cDNA, Filamin 1 and GFP were transcribed with CMV promoter (p), and FILIPs were integrated into expression

vector for mammal cell (pBudCE4; Invitrogen) so as to be transcribed and expressed with EF-1 $\alpha$ promoter (p'), and tranfected into COS-7 cells. Then, they were cultured in the presence or absence of 50 $\mu$ m calpeptine in addition to the conditions of example 1, and expression amount of HA-Filamin 1 and GFP were confirmed by SDS-PAGE method. Further, HA-Filamin 1 and GFP were confirmed that they were translated from the same mRNA in the cell. The relative amount of HA-Filamin 1 that expressed at COS-7 cells in the presence or absence of S-FILIP (S) or L-FILIP (L) was measured on the basis of GFP expression amount. (The relative amount of recombinant Filamin 1 and GFP was 4.7 in the absence of FILIPs and 1.8 in the presence of S-FILIP.) These results are shown in Fig. 3d. This revealed that the expression amount of Filamin 1 was decreased in the presence of FILIPs, especially of L-FILIP. It shows little HA-Filamin 1 protein exists in the presence of mRNA of HA-Filamin 1 and GFP. However, it was suggested that FILIPs induced degradation of Filamin 1, since these effects were lost in the presence of calpeptine which is a protease inhibitor.

Meanwhile, COS-7 cells expressing L-FILIP-GFP were generated in the same manner as mentioned in example 2, and immunoreactivity of them against Filamin 1 was examined. The results are shown in Fig, 3e. Arrows in the figure indicate COS-7 cells expressing L-FILIP-GFP, the scale bar in the figure indicates 25 μm. As a result, at COS-7 cells expressing L-FILIP-GFP, especially the amount of endogenous Filamin 1 was remarkably declined compared with adjacent cells which do not express FILIP. It led to that COS-7 cells expressing L-FILIP-GFP showed law immunoreactivity against Filamin 1. It was also revealed that L-FILIP showed higher activity compared with S-FILIP in degradation of Filamin 1. That is, because S-FILIP does not degrade Filamin 1 abundantly, although most of S-FILIPs in cells colocalize with Filamin 1 and F-actin, the punctate distribution of F-actin, which was observed in COS-7 cells expressing L-FILIP, was also observed even in a small fraction of cells expressing S-FILIP. Moreover, induction of degradation of Filamin 1

protein associated with FILIPs can be thought as one of causes which brings low immunoreactivity against Filamin 1 colocalizing with FILIPs (particularly L-FILIP) as shown in Fig. 2c.

Example 4 (Regulation of cell migration from ventricular zone by FILIPs in developing neocortex)

Since FILIPs introduced into COS-7 cells exert inhibitory effects on cell migration as well as inducing degradation of Filamin 1, when Filamin 1 gene is mutated, postmitotic cells affected by the mutation remain in the ventricular zone, and cause malformation of human cortical. It is likely that FILIPs play a pivotal role in the control of cell migration in the developing neocortex (Neuron 16, 77-87, 1996, Neuron 21, 1315-1325, 1998). Thus, in order to examine the role of FILIPs on neuronal migration in vivo, plasmid DNA (S-FILIPS-GFP cDNA, L-FILIPS-GFP cDNA, or GFP cDNA) was administered into the lateral ventricle of E18 rat brain, then plasmid DNA were incorporated to ventricular zone cells by delivering electrical pulses with a square-pulse electroporator (BEX). The brain of E18 rat was sliced coronally into 200 μm with a microtome, and the dorsal portion of the cortex was dissected out and cultured for four days on a collagen-coated membrane (Transwell®-COL, Costar®-Corning) in a DMEM/F12 medium containing 10% FBS and N2 supplement. After cultured, the obtained cortex sections were fixed with 4% paraformaldehyde/0.1 M PB (pH 7.4) and their images were analyzed on a Zeiss™ LSM510 laser-scanning confocal microscope ( Zeiss™) (Fig. 4a). In Fig. 4a, each of the drawings in the right shows enlarged illustration of inside of the frames in each of the drawings in the left. White dots, p, and V show the edges of the section, pial surface, and lateral ventricle, respectively. Scale bars in Fig. 4a indicate 200 μm (left) and 100 μm (right). The migration rate of each cell against GFP or FILIPs-GFP was obtained by determining the quantity of cells at each site of cortex [cortex was divided equally into five section from lateral ventricle side (VS) to pial surface side

(PS)] which were on the fourth day since they were cultured (Fig. 4b). The value of S-FILIP-GFP was obtained from three sections, while that of L-FILIP-GFP was obtained from five sections and the mean value were calculated as ± s. e. m., respectively.

Consequently, in GFP-expressing cells (GFP) as control, it was confirmed that a number of labeling cells localizing in the ventricular zone was migrating out towards pial surface. These cells were spindle-shaped with leading and trailing processes oriented cortex radially (Neurosci. Res. (Suppl.) 24, S18, 2000). In contrast, cells expressing S- or L-FILIP-GFP were quite different in shape and migration rate from those of GFP-expressing cells only. These expressing cells were round, did not spread long and radially, and hardly migrated staying around neighborhood of the ventricular zone. These effects of FILIPs in ventricular zone cells were consistent with those in COS-7 cells. Fewer cells expressed L-FILIP-GFP compared with GFP or S-FILIP-GFP. The number of cells expressing L-FILIP-GFP did not show significant difference even where they were cultured. These might be caused by the low efficiency of the transfection or translation.

Next, the ontogenetic expression profiles of L- and S-FILIPs in developing rat neocortex were analyzed by immunoblotting with anti-FILIP antibodies. The results are shown in Fig. 4c. The above-mentioned anti-FILIP antibodies (polyclonal anti-FILIP antibody) were prepared with rabbits immunized by synthesized peptide equivalent to the $892^{nd}$ to $909^{th}$ residues of the amino acid sequence of S-FILIP, as the method described in J. Neurochem. 75, 1-8, 2000. From this result, L-FILIP was confirmed more remarkably than S-FILIP in cortex developing process. S-FILIP and L-FILIP appear to play similar roles, however, it is apparent that L-FILIP is the major partner of Filamin 1 in the developing neocortex, since L-FILIP expresses at higher level and shows higher capacity for inducing Filamin 1 degradation. Expression of filips mRNA was low on E18, it is likely that already transcribed FILIP proteins remain in sufficient amounts.

Because filips express in ventricular zone, it is likely that FILIPs interact with Filamin 1 gene and induce the degradation in ventricular zone. Cortical solution of E12 rat [protein solution solubilized with a buffer containing 20 mM TRIS™ (pH 7.5), 150 mM NaCl, 1000 U/ml DNase I, 1% NP-40, 1 mM phenylmethanesulfonyl fluoride, 5 μg/ml aprotinin, 1.5 μM pepstatin A, 2 μM leupeptin] were immunoprecipitated using either anti-Filamin 1 antibodies or anti-c-Myc antibodies (Santa Cruz), and protein was detected using anti-FILIP antibody as a probe. The results are shown in Fig. 4d. The results show that L-FILIP was detected from neocortex solution of E12 rat, while S-FILIP was hardly detected (line 1 in Fig. 4d). Further, since L-FILIP was coimmunoprecipitated with anti-Filamin 1 antibodies in the same solution (line 3 in Fig. 4d), it was revealed that endogenous FILIP (L-FILIP mainly) interacted with endogenous Filamin 1. However, anti-c-Myc antibodies (control) did not show any positive signal (line 2 in Fig. 4d).

It has been known that Filamin 1 protein expresses in migrating and postmigratory neurons in the intermediate zone and the cortical plate of human embryonic brain (Neuron 21, 1315-1325, 1998). Expression of Filamin 1 at rat cerebral cortex was examined with in situ hybridization histochemical study. The results are shown in Fig. 4e. In the figure, CP, S, V, and VZ indicates cortical plate, cranium, lateral ventricle, and ventricular zone, respectively. The scale bar shows 100 μm. The expression of Filamin 1 gene could be confirmed all through the developing cortex, particularly the high expression in ventricular zone was confirmed from these results. Further, expression of the above-mentioned Filamin 1 at cerebral cortex of rat was examined with immunohistochemistry. Frozen sections prepared from E16 rat cerebral cortices fixed with the Zamboni's solution [0.1 M PB (pH 7.4), 2% paraformaldehyde, 0.21% picric acid] were air-dried, permeabilized with PBS containing 0.2% TRITON® X-100, 0.5% bovine serum albumin for 30 min and incubated in the coexistence of anti-Filamin antibodies (1:40; Sigma), followed by incubation in the coexistence of anti-goat IgG antibodies bound with fluorescein (1:100;

Jackson ImmunoResearch Laboratories) and stained. The results are shown in Fig. 4f. In the figure, CP, V, and VZ indicate cortical plate, lateral ventricle, and ventricular zone, respectively. The scale bar shows 100 μm. It was revealed by these results that ventricular zone cell highly expressed Filamin 1 gene, while Filamin-like immunoreactivity was lower than those observed in the intermediate zone and cortical plate. Since Filamin 1 is closely related to cell migration (Science 255, 325-7, 1992, Neuron 21, 1315-25, 1998), it is likely that degradation of Filamin 1 in the ventricular zone through the action of FILIPs is a significant process for controlling the start of migration. The process is a unique molecular mechanism of inhibitory control over radial migration of cells out of the ventricular zone during developing cortex.

**INDUSTRIAL APPLICABILITY** The proteins having effects of controlling cell migration and cell death and DNA encoding the proteins are control molecules of cytoskeletal protein. Therefore, they are applicable for an inhibitor for metastasis of a cancer or a tumor or a regulant of cell migration for transplantation treatment as well as for controlling cell motility and cell death. They are further applicable for controlling cell motility and cell death, screening for promoters or inhibitors of effects of controlling cell migration and/or cell death, and promoters or inhibitors for expressing the proteins/peptides of the present invention, and etc. by using the aforementioned proteins having effects of controlling cell motility and cell death and DNA encoding the proteins. Furthermore, using the proteins/peptides of the present invention makes it possible to reveal the mechanisms of metastasis of a cancer or a tumor, cell migration of neuron and the like.